Qualitative risk assessment of introduction of anisakid larvae in Atlantic salmon (*Salmo salar*) farms and commercialization of products infected with viable nematodes

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ABSTRACT

In this study, the likelihood of introduction of anisakid larvae in Atlantic salmon (*Salmo salar*) farms and subsequent commercialization of fish infected by at least one vital parasite was formally assessed using an adaptation of the risk assessment framework for importation of animals and animal products of the World Organization for Animal Health (OIE). The different plausible pathways were identified and outlined. The most recent information concerning the parasite, its definitive, intermediate hosts and the farming practices typical of Atlantic salmon farms were reviewed and used to assess the likelihoods of each key step along the pathways. A matrix for the conditional probabilities was adopted to combine the qualitative estimations and obtain an objective and transparent overall risk of introduction along each route. In order to avoid misinterpretation and overconfidence on the outcome, the uncertainties surrounding the estimations were considered. The only situation for which the assessed risk in a typical Atlantic salmon farm was deemed to be non-negligible involved the ingestion of infected hosts that penetrate the harvesting cages. In this event, the overall risk was estimated as 'Very Low' with a high degree of uncertainty because of the scarcity of information in some of the key steps along the pathway. However, the scientific evidence in support of the overall estimation suggests that the availability of additional data would be unlikely to increase the final estimated risk. On the basis of the available information of the system, the estimated risk of introduction and commercialization of farmed Atlantic salmons infected by viable nematodes resulted 'Very Low' even under a conservative approach. The proposed qualitative model is an objective and transparent method to assess the risk when data and information are scarce and provides a framework for the qualitative assessment of the introduction of alive parasites in aquaculture/mariculture implants and subsequent commercialization of infested fishery products. The framework could be easily adapted to other parasite-host interactions besides anisakid nematodes in Farmed Atlantic salmons.

1. INTRODUCTION

Fish-borne parasitic zoonoses represent a global emergent threat, among these, anisakidosis has shown widespread expansion in the last two decades (Chai, Darwin Murrell, & Lymbery, 2005). The family Anisakidae includes zoonotic parasitic nematodes among which, the species belonging to the genera *Anisakis* and *Pseudoterranova* are the most commonly associated with infection in humans due to consumption of raw or undercooked fishery products. As a result of their impact on the commercial value of fishery products and on public health, anisakids have become an important economic and a public health concern worldwide (M. T. Audicana & Kennedy, 2008; Bouree, Paugam, & Petithory, 1995; EFSA, 2010; Skirnisson, 2006).

The life cycle of Anisakidae of zoonotic interest is completed in seawaters and involves marine mammals (cetaceans and pinnipeds) and piscivorous birds as definitive hosts. In natural conditions, the predation of infected fish leads to bioaccumulation along the predation chain resulting in the risk of infection being higher in top predator fish species (Strømnes & Andersen, 2003a) which as a result pose a higher risk for human health.

In order to prevent and control transmission of fishery product-borne parasites, the Section VIII of Annex III to Regulation (EC) No 853/2004 lays down provisions for fishery products to be consumed raw or almost raw. The Regulation indicates that fishery products intended to be eaten after a process that is not sufficient to inactivate nematode larvae must be frozen at a temperature of not more than -20°C in all parts of the product for not less than 24 hours.

In April 2010, the European Food Safety Authority (EFSA) published a scientific Opinion on risk assessment of parasites in fishery products (EFSA, 2010) providing criteria for determining the conditions under which fishery products from aquaculture can be recognized as being free of viable parasites that may represent a hazard for human health. With particular reference to farmed Atlantic salmon (*Salmo salar*), the Opinion concluded that farmed Atlantic salmons reared in floating cages or onshore tanks and fed with compound feedstuffs are unlikely to contain live parasite. However, the Panel on Biological Hazards did not considered routes of infection other than the feed and the risk was never assessed formally.

Following that Opinion, in 2011, the Regulation (EC) No 1276/2011 modified the requirements set out in Annex III, Section VIII, Chapter III, Part D of Regulation (EC) No 853/2004 allowing food business operators to not apply freezing treatment if procedures approved by the competent authority are used to verify that the product does not represent a health hazard with respect to viable parasites.

In the present study, the general approach recommended by the World Organization for Animal Health (OIE) for the assessment of the risk posed by the importation of live animal and animal products (OIE, 2010) was adapted to formally investigate the potential for live zoonotic nematodes to represent a risk for human health in farmed Atlantic salmon.

2. MATERIALS AND METHODS

For purpose of this study, the approach proposed by the OIE for the assessment of risks associated with the importation of animals or animal products (OIE, 2010) was adopted. According to the OIE guidelines, the identification of the hazard precedes the risk assessment, which is composed of three components: (i) Release assessment, (ii) Exposure assessment and (iii) Consequence assessment. Generally, the final risk estimate is the result of the integration of the three steps but because of the purpose of this study, the consequences of the commercialization of the infected fish are not considered. The risk question being assessed was therefore formulated as "what is the likelihood of an Atlantic salmon (*Salmo salar*) harvested from a typical farm being infected by at least one nematode of genus *Anisakis,* and subsequently commercialized for human consumption".

The different pathways describing the sequence of sufficient and necessary events leading to the introduction of the parasite into a fish farm were outlined and the likelihoods of introduction assessed for each pathway considering the farming practices typical of Atlantic salmon.

Qualitative risk assessment models foresee the use of subjective risk levels to describe the likelihood of unwanted events; in this work, the qualitative terms proposed by Kahn et al. (S.A. Kahn et al., 1999a; S.A. Kahn, Wilson, Perera, Hayder, & S.E., 1999b) were adopted (Table 1).

Table 1 Definition of the qualitative terms used to describe the likelihoods of the necessary events leading to the introduction of anisakid nematodes into an hypothetical Atlantic salmon farm and commercialization of infested products (after Kahn et al.1999).

Likelihood	Decription
High (H)	Expected to occur
Moderate (M)	Occurrence less than 50% probability
Low (L)	Unlikely to occur
Very low (VL)	Rarely occur
Extremely low (EL)	Very rarely occur
Negligible (N)	Chance of occurrence so small that can be ignored

The biological and epidemiological characteristics of the parasite, its primary and accidental hosts together with the biosecurity practices applied in Atlantic salmon's farms were reviewed and discussed to assign the likelihood at each step along each identified pathway. Information and evidence included in this assessment were collected from peer-reviewed articles and from industry data reported in the environmental public reports published by the main producers of farmed Atlantic salmon worldwide. In each pathway, the likelihoods assigned to each event were combined to derive the overall estimate of the risk of introduction of the parasite in farmed salmon and commercialization of infested products. The risk estimates were expressed as cumulative likelihoods obtained combining the qualitative estimates of the inputs according to the matrix of conditional probabilities presented in table 2 and previously applied by EFSA and in other qualitative risk assessments (EFSA, 2007; Peeler & Thrush, 2009).

Table 2 Combination matrix used for the estimation of the conditional likelihoods. The product of two probabilities is always less than the lowest probability and is sometimes given as a range (e.g. N-EL). However, as explained in the EFSA report, since qualitative terms cover a wide range of likelihoods, the combined estimate is, in some cases, equal to the lower estimate (e.g. a step 'n' with an estimate of VL with a step 'n+1' with an estimate of EL produces and an overall estimate of N-EL).

Likelihood step '*n+1*'	Conditional likelihood step *'n'*					
	N	**EL**	**VL**	**L**	**M**	**H**
H	N	EL	VL	L	M	M
M	N	EL	VL	VL	L	M
L	N	EL	EL	VL	VL	L
VL	N	N-VL	EL	EL	VL	VL
EL	N	N	N-EL	EL	EL	EL
N	N	N	N	N	N	N

This matrix defines a likelihood estimate for any binary combination of conditional events. If two or more independent risk factors contributed to the likelihood estimation for a single step, the likelihoods for each factors were estimated and the same matrix for the conditional probabilities was used to outline the overall likelihood for the step.

In order to avoid overconfidence on the outcomes and prevent misinterpretation, an assessment of the uncertainty surrounding each estimation was also carried out (Table 3), and expressed as: High, (H) Moderate (M) or Low (L).

Table 3 Definition of the qualitative terms used to define the levels of uncertainty surrounding the likelihoods assigned to the steps along the pathways.

Uncertainty	Interpretation
Low (L)	The estimation is strongly supported by data-evidence. Agreement by different authors
Medium (M)	The estimation is supported by few or incomplete data. Some authors report slightly different conclusions to others
High (H)	The estimation is supported only by scarce data or it is based on hypotheses not yet proved. Strong disagreement from different authors

With respect to the uncertainties, the worst estimate was conservatively considered among the risk factors and along the steps of the pathways; in this way, a high uncertainty in one level is enough to lead to a high uncertainty in the overall outcome. An exception was made if the occurrence of the event in step n+1 is *Negligible* with *Low* uncertainty.

2.1 Hazard identification and characterization.

The different species belonging to genera *Anisakis* and *Pseudoterranova* are not reliably distinguishable morphologically but several species have been identified at molecular level (S. Mattiucci et al., 1997; Simonetta Mattiucci et al., 2005; Paggi et al., 2000; Paggi et al., 1991). The morphospecies most commonly associated to human infection are:

(i) *Anisakis simplex,* worm-like parasite, with larvae usually measuring 1 - 3cm length, thin, characterized by a pinkish-white colour. It usually appears rolled up on itself. Larvae are normally localized in the viscera where they are generally easily visible but can migrate into the muscle or the abdominal wall where the parasite is more difficult to identify, especially in white fish (EFSA, 2007);

(ii) *Pseudoterranova decipiens*, worm-like parasite, larvae usually measuring 1 - 4cm length and characterized by a reddish-brown colour it tends to present a large-rolled coil. In infested specimens it is usually located at muscular level (McClelland, 2002).

The life cycle of the species belonging to genus *Anisakis* takes place in seawater and proceeds in several steps. In the first step, eggs, are released in seawater with the faeces of the definitive hosts (mainly cetaceans such as whales, dolphins and porpoises); in marine environment they develop to second stage larva and hatched in seawater (Anderson, 2000; Køie, Berland, & Burt, 1995; McClelland, 2002; Smith & Wootten, 1978).

Newly hatched larvae can survive in marine environment for weeks and be eaten by a wide range of different intermediate hosts (crustaceans and molluscs) where they develop to third stage larvae (L3). When fish or cephalopods eat intermediate infected hosts, the L3 migrates to the coelomic cavity of the predator, which acts as paratenic host since the parasite does not further develop. Paratenic hosts can acquire larvae by ingesting intermediate and paratenic hosts leading to bioaccumulation along the predation chain; consequently, big and/or old fish and at the top of food chain may host even thousands of nematodes (Smith & Wootten, 1978). Humans act as accidental hosts when they eat undercooked infected fish or squid.

The larval stages and the biological cycle of *Pseudoterranova spp.* do not differ from the ones described for *Anisakis spp.* even though the definitive hosts are usually pinnipeds like sea lions or seals instead of cetaceans. Moreover, larvae of *Pseudoterranova.spp,* do not have cuticular sheaths with lateral extremities that increase the buoyancy, thus, conversely to larvae of *Anisakis spp.*, are not able to swim (Palm, 1999).

Although the dynamics underlying the geographical distributions of the most important intermediate and paratenic hosts of *Anisakis spp.* and *Pseudoterranova spp.* are complex and still largely unknown (EFSA, 2010), considering the differences in the habitat of the hosts involved; it is generally recognized that *Anisakis spp.* have an essentially pelagic life-cycle, whereas *Pseudoterranova spp.* have a more benthic habit. Consequently, with particular reference to the Atlantic salmon, parasites belonging to the genus *Anisakis spp.* represent a greater concern than *Pseudoterranova spp. (Wootten, Yoon, & Bron, 2010).*

Following these considerations, nematodes of genus *Anisakis* were formally identified as the hazard of interest while *Pseudoterranova spp.* is not considered further.

2.1.1. Hazard characterization *Anisakis spp.* – Prevalences.

The prevalences of the parasite in different wild fishes and data related to the occurrence of the different species of genus *Anisakis* in infected fish show high variability according to both geographical region and hosts species (EFSA, 2010; Mladineo & Poljak, 2014; Piras et al., 2014; Karl Marx A. Quiazon, Tomoyoshi Yoshinaga, & Kazuo Ogawa, 2011). The complexity of the dynamics of infection leading to different proportions of the parasite's species in different hosts and in different areas led to the cautionary conclusion that none of the fishing areas worldwide should be considered as *Anisakis*-free, and thus, all the wild saltwater fishery products must be considered potentially infested (EFSA, 2010). For this reason, geographical heterogeneities were not considered in this assessment.

2.1.2. Hazard characterization *Anisakis spp.* – Pathogenesis.

In humans, the accidental ingestion of live nematodes belonging to the family of Anisakidae, causes parasitic zoonosis known as anisakidosis or anisakiasis, described for the first time in 1960 by Van Thiel (Van Thiel, Kuipers, & Roskam, 1960). The minimum infectious dose is a single nematode (A. Daschner, Alonso-Gomez, Cabanas, Suarez-de-Parga, & Lopez-Serrano, 2000; FDA, 2012). After ingestion, viable larvae may be excreted with the faeces after up to 48 hours with the faeces, or result in the acute form of anisakidosis, the most frequently observed form, characterized by violent abdominal pain, nausea and vomiting due to the larval inflammation of the intestinal mucosa. (Asaishi, Nishino, Totsuka, Hayasaka, & Suzuki, 1980; Sakanari & McKerrow, 1989; Sugimachi, Inokuchi, Ooiwa, Fujino, & Ishii, 1985). The acute form might degenerate into chronic if misdiagnosed or untreated. In the chronic form, the larvae penetrate the gastrointestinal mucosa, causing the formation of granulomas with eosinophilic infiltrate and even abscesses. Granulomas and the inflammation process remain even after the death of the worm that in the human body usually happens 3 weeks after ingestion. Complications have rarely been reported in the literature and include a few episodes involving intestinal obstruction (Sasaki, Fukumori, Matsumoto, Ohmori, & Yamamoto, 2003), colic intussusception (Furukawa et al., 2014;

Yorimitsu et al., 2013) and pneumoperitoneum (Ito et al., 2007). Moreover, the consumption of fish harbouring dead *Anisakis spp.* larvae has been reported to be potentially dangerous because of possible allergenic reactions (M. a. T. Audicana, Ansotegui, de Corres, & Kennedy, 2002).

2.2 Release and Exposure assessment for the Introduction of *Anisakis spp* into an Atlantic salmon farm

The risk of the introduction of *Anisakis spp.* into an Atlantic salmon farm was assessed considering five pathways:

1. *Introduction through juveniles of wild Atlantic salmon captured for farm production*
2. *Introduction through feed contaminated with viable Anisakis spp. larvae*
3. *Introduction through wild salmon that have accidentally entered the floating cages*
4. *Introduction through escaped salmon that have been infected offshore re-entering the cages.*
5. *Introduction through the ingestion of infected hosts*

For each pathway the sequence of sufficient and necessary events leading to the release of the parasite into a generic farm were identified and are outlined in figures 2-5.

2.2.1 Pathway 1: Introduction through juveniles of wild Atlantic salmon captured for farm production

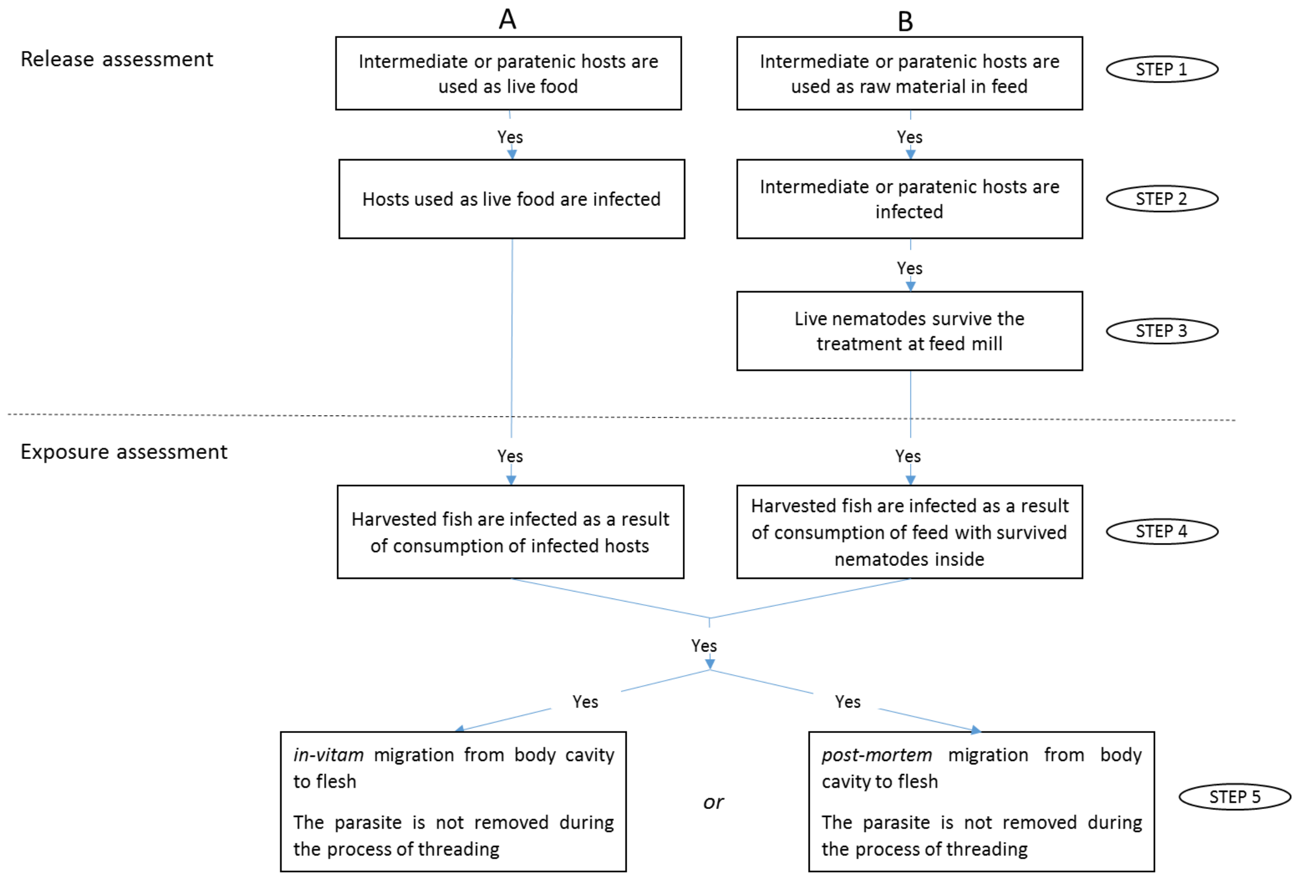
The occurrence of anisakid larvae in wild salmon is known to be high and above 70% (EFSA, 2010; FDA, 2015), therefore, the harvest of wild animals could represent an important pathway for the commercialization of risky products. However, unlike the farming methods applied for other species (i.e. Cod or Eels), the production cycle of Atlantic salmon is totally closed and the capture of juveniles does not occur. This pathway was not further explored.

2.2.2 Pathway 2*:* Introduction through feed contaminated with viable *Anisakis spp*. larvae

The likelihood of the introduction of the parasite into a hypothetical farm trough the feed depends on: (i) the source and the nature of the raw material and (ii) the thermal/physical treatments to which raw material has been subjected.

The scenario trees outlined in Figure 1 represent the pathways leading to the introduction of the parasite into a generic farm by feed. Both the use of live feed (A) and treated feed (B) were considered.

Figure 1. Pathway nr.2; flowcharts outlining the required steps for the introduction of Anisakis spp. into a hypothetical farm through feed. Both live food (pathway A) and feed were considered (pathway B).



The use of live feed would lead to an evident risk of introduction of the parasite (Wootten & Smith, 1975), however, the farming of the Atlantic salmon foresees the use of heat-treated feed only; therefore, the pathway A was not considered. Moreover, since farmed salmon are fed with composite feed which includes wild species like herring (*Clupea harengus*), capelin *(Mallotus villosus)*, Chilean anchovies *(Engraulis ringens)* etc., the first step of pathways B was considered as an event that always occurs, and its likelihood was not included in the assessment.

Step 2. Considering the wide range of intermediate/paratenic hosts species to which *Anisakis spp.* have adapted (EFSA, 2010; Klimpel, Palm, Rückert, & Piatkowski, 2004), together with the variability and the uncertainty underlying the presence of the parasite in wild species (Section 2.2.1); the likelihood of *Anisakis spp.* being present in wild species used as raw material for the farmed salmon feed is considered ***High*** with ***Low*** uncertainty.

Step 3. Farmed salmon are fed with dry pellet produced by extrusion and temperature above 150°C. The likelihood of parasite surviving the treatment is ***Negligible*** with ***Low*** uncertainty.

Step 4. Following the processing treatments at feed mill, the next hypothetical step is the consumption of feed containing viable larvae. The conservative likelihood assigned to this step is ***High*** with ***Low*** uncertainty.

Step 5. At this stage, the combined likelihood of the parasite migrating (*intra-vitam* and/or *post-mortem*) from the coelomic cavity to edible muscles and not being removed during the process of threading is assessed.

The *intra-vitam* migration of the parasite is not a certain event and the frequency distributions of anisakid third stage larvae in hosts' tissues are believed to be affected by a number of conditions encountered within the hosts themselves (Strømnes & Andersen, 1998) among which, the lipid content is believed to play an important role (Strømnes, 2014; Strømnes & Andersen, 2003b).

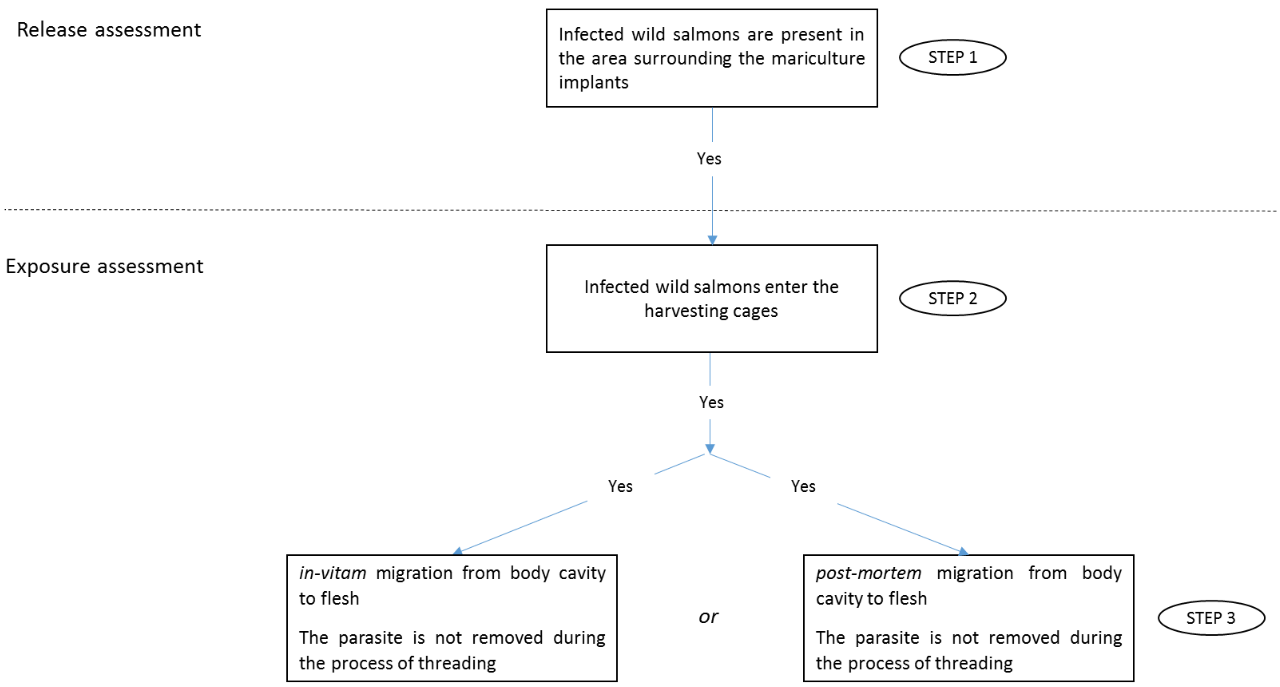
Several recent studies reported the presence of anisakid nematode larvae in muscles surrounding the body cavity of freshly caught salmonids (Karl, Baumann, Ostermeyer, Kuhn, & Klimpel, 2011; Senos, Poppe, Hansen, & Mo, 2013; Setyobudi, Jeon, Lee, Seong, & Kim, 2011; Urawa & Fujisaki, 2006) or sibling species (Karl Marx A Quiazon, Tomoyoshi Yoshinaga, & Kazuo Ogawa, 2011) indicating that the *intra-vitam* migration of the parasite is an event that it is likely to occur in salmonids. Following these considerations, the estimated likelihood of parasite *intra-vitam* migration from the coelomic cavity is considered to be ***High,*** with ***Low*** uncertainty.

The *post-mortem* migration of the parasite from the viscera to flesh is still a debated topic, the scientific opinion from the Panel on Biological Hazards (EFSA, 2010) reported: "… based on scientific evidence it is not clear when, under what conditions and in which fish species, *post-mortem* migration of *Anisakis simplex* larvae occurs …". However, factors stimulating the migration of the parasite after the death of the host are presumably related to physico-chemical changes in viscera (Smith & Wootten, 1975) and time-temperature storage conditions (Chen, Cheng, Grabner, Chang, & Shih, 2014; Cipriani et al., 2015). At this respect, it should be considered that opposite to wild salmons, reared fishes are processed immediately after collection from the floating cages; consequently, the likelihood of *post-mortem* migration in farmed Atlantic salmon is considered as ***Negligible*** with ***Low*** uncertainty. The worst scenario (intra-vitam migration) was conservatively considered in this step.

2.2.3 Pathway 3: Introduction through wild salmons that have accidentally entered the floating cages

The pathway leading to the introduction of *Anisakis spp.* by the accidental entry of wild salmons into the floating cages is outlined in Figure 2.

Figure 2 Pathway nr.3; flowchart outlining the required steps for the introduction of Anisakis spp. into a hypothetical Atlantic salmon farm and commercialization of infested products by accidental entry of wild salmons in floating cages.



Step 1. As mentioned in section 2.2.1. The occurrence of *Anisakis* in wild salmon is known to be high; however, although the presence of wild salmons in the areas surrounding the salmon farms cannot be excluded, a low density of wild salmon is usually recorded in the areas bordering the mariculture implants (Ford & Myers, 2008; Frazer, 2009). From these evidence, the estimated likelihood of wild salmons being present in the area surrounding the farm is ***Low*** whilst the likelihood of wild salmon being infected if present is ***High;*** consequently, the combined likelihood for the presence of infected wild salmons in the area surrounding the farms is ***Low***. Because of the scarcity of available data to assess the salmon distribution variability across the geographical areas, the level of uncertainty was considered ***Medium*** for the first condition and ***Low*** for the second one leading to an overall conservative ***Medium*** level of uncertainty for this step.

Step 2. Atlantic salmons are grown to marketable size in floating nets offshore; the possibility for a wild salmon to penetrate the harvesting nets and to mingle with the reared salmons is linked to the presence of a hole in the floating cages. However, the firsts consequences of a hole in a floating net would be the escape of the raised fish, with obvious economical loss and huge environmental consequences (Crozier, 1993; Fraser, Cook, Eddington, Bentzen, & Hutchings, 2008; Gausen & Moen, 1991; Thorstad et al., 2008); consequently, it is of industry interest to apply all the biosecurity measures aimed to prevent/avoid the escape of the reared fishes, and indirectly, the introduction of wild animals. At this respect, the major food business operators in salmon harvesting invest many resources to pursue the so-called 'zero escape' objective and public reports shown how the efforts resulted in a steadily decreasing occurrence of incidents leading to 'escape' events (Lerøy Seafood Group, 2012, 2013, 2014; Marine harvest, 2012, 2013, 2014).

Moreover, incidents resulting in an escapes should not be interpreted as events favouring at the same time the introduction of wild individuals. In fact, all the reported incidents were one-way oriented in determining the 'escape' without favouring the 'introduction' in any way.

In order for an introduction to take place, any breakage in the netting would need to be of a size insufficient to allow a large-scale release of farmed fish, which would be noticed immediately by the operators on underwater surveillance cameras. Conversely, this underwater surveillance system reduces the likelihood of ingress of wild salmon to the cages, since any damage in netting is likely to be relatively small in size.

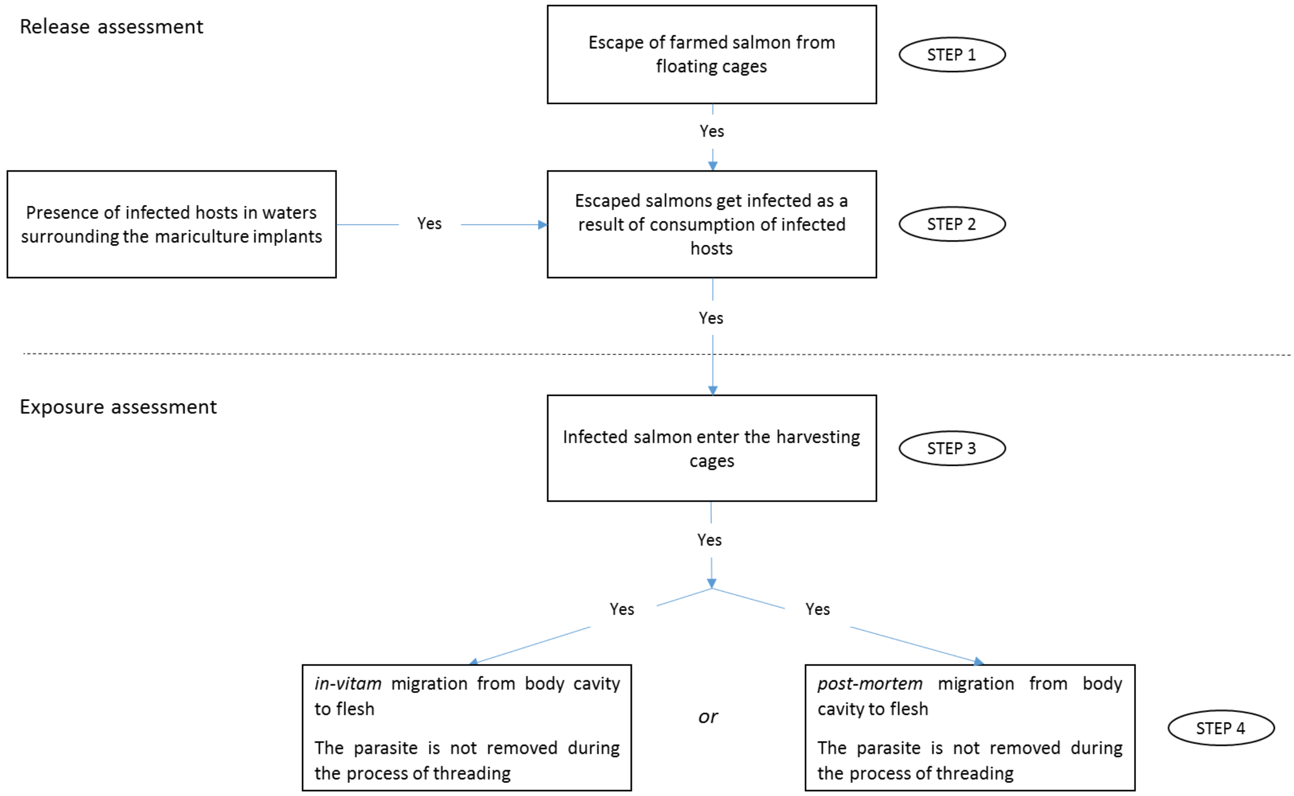
Following these considerations, the estimated likelihood of the wild salmon accidentally penetrating the floating cages is ***Negligible*** with ***Low*** uncertainty.

Step 3. Following the considerations in Section 2.2.2. (Step 5), the estimated likelihood of anisakid larvae migrating in flesh from the coelomic cavity is ***High*** with ***Low*** uncertainty**.**

2.2.4 Pathway 4: Introduction through escaped salmon that have been infected offshore re-entering the cages.

Although the 'escape' events are rare (Section 2.2.3, Step 2), the likelihood of the re-introduction of escaped salmon that have been infected offshore is assessed (Figure 3).

Figure 3 Pathway nr.4; flowchart outlining the required steps for the introduction of Anisakis spp. into a hypothetical Atlantic salmon farm and commercialization of infested products by escaped salmon that have been infected offshore re-entering the cages.



Step 1. From consideration discussed in Section 2.2.3. (Step 2), the estimated likelihood of farmed Atlantic salmons escaping in seawaters is ***Low*** with ***Low*** uncertainty.

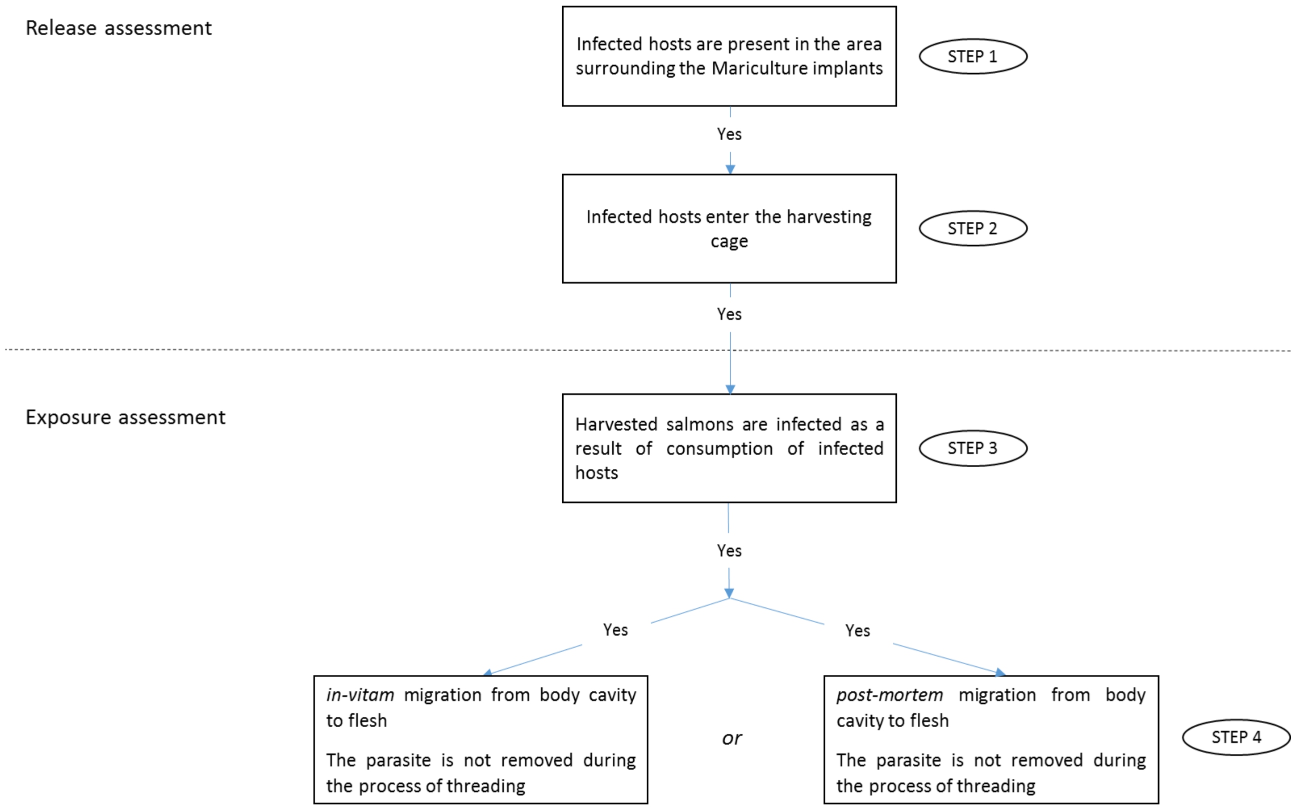
Step 2. *Anisakis spp.* larvae can survive in seawaters for extended periods and be eaten by a wide variety of different hosts. Although the parasite mainly uses *euphausiids* (krill) living in deeper water offshore as first intermediate host (Smith, 1983), the parasite is able to select host species depending on the locality (Klimpel et al., 2004). Therefore, the estimated likelihood for the presence of infected hosts in the areas bordering the implants is ***High***, with a ***Low*** uncertainty. Since escaped salmons are forced to prey to survive, the estimated likelihood of escaped salmons preying infected hosts is ***High*** with ***Low*** uncertainty.

Step 3-4. Estimated likelihoods and uncertainties for these steps are identical to the ones reported in pathway 3.

2.2.5 Pathway 5: Introduction through the ingestion of infected hosts

The pathway leading to the introduction of *Anisakis spp.* by ingestion of infected hosts is outlined in Figure 4.

Figure 4. Pathway nr.4; flowchart outlining the required steps for the introductio. n of Anisakis spp. into a generic farm by ingestion of infected hosts.



Step 1. Following considerations in Section 2.2.4. (Step 2), the estimated likelihood of infected hosts being present in areas bordering the implants is ***High*** with ***Low*** uncertainty.

Step 2. The access of infected hosts to the floating cages is strictly dependent on the size of the hosts themselves. In fact, while the introduction of large hosts is physically prevented by the meshes' size, no barriers are applicable to hosts smaller than the meshes. Thus, the estimated likelihood for this step is ***Medium***, with a ***High*** level of uncertainty due to the lack of information about the occurrence of the different host species (with particular interest in host size) in the area bordering the implants.

Step 3. In this step, the ingestion of anisakid larvae is linked to the predation of the hosts. Even though farmed salmon are fed with dry pellet, the results of two recent studies (Marty, 2008; Mo et al., 2014) reported the presence of *Anisakis spp.* larvae in runts of farmed Atlantic salmon (Fishes with clear signs of poor performance and/or abnormal appearance, emaciated and not suitable to be marketed for human consumption), suggesting that farmed salmonids in open cages may in some cases feed on live food. However, it is important to emphasize that the nematodes were found so far only in underweighted or discarded animals and never in salmons processed for human consumption. The authors explained their findings hypothesizing that in floating cages weak animals undergo competition phenomena that limit their access to feed and thus, runts must feed with ‘anything’ that can be eaten in order to survive. Based on this evidence, it is assumed that the likelihood of salmons processed for human consumption (i.e. not classified as ‘runts’) preying live food to supplement their feed intake is ***Low***. *A* ***High*** uncertainty is cautiously assigned because of the scarcity of data supporting the assessment.

Step 4. As discussed in Section 2.2.2 (Step 5), the presence of the parasite in infected salmons represents a risk for human health only if the parasite is not physically removed during the process of threading. According to previous estimations, the likelihood of the event is ***High*** with ***Low*** uncertainty.

3. RESULTS

The estimated likelihoods and uncertainties for each step combined through the Combination matrix (Table 2) provided the cumulative likelihoods; these are reported for each of the pathways considered in tables 4-7 with estimated likelihoods and uncertainties in brackets.

Table 4 Summary of risk estimates for pathway 2: 'Introduction through feed contaminated with viable Anisakis spp. larvae' (H=High, M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible).

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Intermediate or paratenic hosts are used as raw material for the feed	Certain event	
2	Hosts used as raw material for the feed are infected	H	L
3	The parasite survive the treatment in feed mill	N (N)	L (L)
Exposure assessment			
4	Survived larvae are ingested	//	//
5	The parasite is not removed during the process of threading	//	//

Table 5 Summary of risk estimates for pathway 3: '*Introduction through wild salmon that have accidentally entered the floating cages*' (H=High, M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible)

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Wild infected salmons are present in the area surrounding the farm	L	M
Exposure assessment			
2	Infected wild salmon enter the floating cage	N (N)	L (L)
3	The parasite is not removed during the process of threading	//	//

Table 6 Summary of risk estimates for pathway 4: 'Introduction through escaped salmon that have been infected offshore re-entering the cages.' (H=High, M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible)

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Escape of farmed salmons	L	L
2	Escaped salmons prey infected hosts	L (H)	L (L)
Exposure assessment			
3	Escaped salmons get infected and re-enter the floating cages	N (N)	L (L)
4	The parasite is not removed during the process of threading	//	//

Table 7 Summary of risk estimates for pathway 5: 'Introduction through the ingestion of infected hosts' (H=High, M=Medium, L=low, VL=Very Low, EL= Extremely Low N=Negligible).

Step	Description	Likelihood (conditional)	Uncertainty
Release assessment			
1	Infected hosts are present in the area surrounding the farm	H	L
2	The infected hosts penetrate the floating cages.	L (M)	H (H)
Exposure and establishment assessment			
3	Infected hosts in floating cages are eaten by high quality harvested salmons	VL (L)	H (H)
4	The parasite is not removed during the process of threading	VL (H)	H (L)

In pathway 2, having assessed the likelihood for step 3 as **Negligible** with **Low** uncertainty, the assessment of the cumulative likelihood did not continued beyond that stage and for the same reason, the assessment along pathways 3 and 4 did not continue beyond steps 2 and 3 respectively.

4. DISCUSSION

In our study, the estimated cumulative likelihoods defined the risk of introduction of *Anisakis spp*. into Atlantic salmon farms (and commercialization of infected products) as *Negligible* or *Very Low* depending on the considered pathway. Our formal qualitative estimations agreed with the available scientific evidence (Angot & Brasseur, 1993; EFSA, 2010; Lunestad, 2003; Skov, Kania, Olsen, Lauridsen, & Buchmann, 2009; Wootten et al., 2010) who generally considered the presence of vital anisakid larvae in farmed salmon as a very unlikely event.

With respect to the second pathway, our estimation coincides with the conclusions reported by EFSA, (EFSA, 2010) and the outcome was characterized by a Low level of uncertainty, indicating strong evidence in support of the result. In fact, to date, there are no evidence or reported cases indicating that nematodes of genus *Anisakis spp.* are able to survive the processes at which the raw materials are subjected (Angot & Brasseur, 1993; Bristow & Berland, 1991; Inoue, Oshima, Hirata, & Kimura, 2000; Lunestad, 2003). Recently, some proteins attributable to *Anisakis simplex* have been found in processed fish products (Fæste, Plassen, Løvberg, Moen, & Egaas, 2014), but the risk related to allergic reactions due to the presence of heat-resistant proteins (Alvaro Daschner, Cuéllar, Sánchez-Pastor, Pascual, & Martín-Esteban, 2002; Pravettoni, Primavesi, & Piantanida, 2012), was beyond the scope of this study.

The cumulative likelihood obtained for the pathway 3 and 4 led to a N*egligible* risk of introduction and this would not change even if further evidence led to the revision of the likelihood estimation for the first step of pathway 3, (characterized by *Medium* uncertainty), to *High*.

The cumulative likelihood of the pathway 5 was considered to be *Very Low* but this outcome was characterized by *High* uncertainty.

The route of introduction by ingestion of infected hosts, although characterized by high uncertainty, was the only pathway leading to an overall estimation of the risk as greater than 'Negligible' and our formal findings seem to support the hypothesis of the authors who recovered larvae of *Anisakis simplex* from farmed salmons (Marty, 2008; Mo et al., 2014). Although characterized by a high level of uncertainty (because of the uncertainty in steps 2 and 3), the lack of evidence found in the literature in support of this route is in agreement with our overall estimate, consequently, it can be hypothesized that the high uncertainties in steps 2 and 3 are the result of lack of data and that it is unlikely that if further data become available the estimation would move upward (i.e. greater than *'Very Low'*).

On the basis of the current knowledge of the biology of the system, and the typical practices adopted in the Atlantic salmon farming, the overall risk of commercialization of product infested by viable larvae appears to be very low despite the overall conservative approach adopted.

Main assumptions and limitations.

As outlined by the hazard identification, the assessment was made considering nematodes belonging to the genus *Anisakis* without distinguishing between the different species, thus, similar properties amongst the species of genus *Anisakis* were assumed. Moreover, it should be noted that because of the differences in the typical habitat between *Anisakis spp.* and *Pseudoterranova spp.*, together with the likelihood of parasite's migration from the intestine to the flesh being considered cautiously high with low uncertainty (making the different migration behaviour of the species no more significant), the results obtained for *Anisakis spp.* could be reasonably extended to *Pseudoterranova spp.*

As infected salmons do not die or appear as unhealthy/unfit for human consumption, in the exposure assessment it was assumed that in the processing line at farm level infected salmons are not recognized.

In the study, only plausible routes of introduction were considered in the release and exposure assessment; nevertheless, since science cannot prove that a particular pathway does not exist there will always be a degree of uncertainty.

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